analysis for creatinine and the TIINE peptide and stored at −70ºC until analysis.
Results: Method performance and validation characteristics were established for the immunoaffinity LC-MS/MS assay aimed at measuring the 45-mer with 5HyP in human urine. Validation experiments were designed to address specific challenges related to quantification of endogenous analytes. The validated method has been shown to be sensitive (lower limit of quantification (LLOQ) 0.156 ng/mL), selective, accurate (<15% RE), and precise (<15% CV) over a linear range of 0.156−7.5 ng/mL. The recovery of the 45-mer peptide from the urine samples of 6 subjects was ranging from 94.2%-108%, which indicated that the recovery was urine matrix independent. Accuracy and precision of spiked dilution linearity samples at 2, 4, 10, and 20-fold dilutions were <15% RE and <15% CV, respectively. Sample stability and inter- and intra-subject variability have been evaluated in the urine of normal and OA populations. The method has been applied to analyze human urine samples from clinical studies. Urinary TIINE 45-mer was consistently found to be elevated in symptomatic patients with X-ray OA in the knee or hip (135±9 ng/mM creatinine) as compared to levels in non-OA subjects (65±12 ng/mM), indicating increase type II breakdown. Moreover, urinary TIINE 45-mer was found to be inhibited in healthy volunteers upon administration of MMP inhibitors in a dose- and time-dependent manner.
Conclusions: The immunoaffinity LC-MS/MS assay to quantify the most abundant urinary type II collagen neoepitope peptide provides highly accurate and precise concentration determination with minimal sample preparation and has been successfully employed in clinical studies aimed at validating the 45-mer TIINE peptide as a biomarker for MMP activity and its role in osteoarthritis.

I-30 MICROARRAY GENE EXPRESSION PROFILING OF HUMAN OSTEOARTHRITIC BONE SUGGESTS ALTERED BONE REMODELLING, WNT AND TGF BETA/BMP SIGNALING

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Purpose: In addition to degenerative changes in the articular cartilage, osteoarthritis (OA) is characterised by alterations to subchondral bone. Changes to bone in OA, which include increased bone volume fraction and reduced bone mineralisation, have also been identified at sites distal to the affected joint. Altered bone remodelling has been proposed to underlie these bone changes in OA. To investigate the molecular basis for these changes, we performed gene expression analysis in bone from the proximal femur of OA and control individuals.
Methods: Messenger RNA prepared from cancellous bone samples, obtained from the proximal femur, were used for both targeted gene analysis, investigating genes thought to be centrally involved in bone turnover, and gene microarray, using Compugen human 19K-oligo microarray slides. In the latter experiments, we compared the gene expression profiles of four sets of OA, CTL and OP bone samples (40 comparisons in total), comprising 10 OA – CTL female, 10 OA – CTL male, 10 OA – OP female and 10 OP – CTL female sample pairs.
Results: We identified clear expression differences between genes such as osteocalcin, osteopontin and type I collagen, between control and OA bone. In the microarray experiments, a large number of differentially expressed genes was identified, and twenty-five of these genes were confirmed to be differentially expressed ($p < 0.01$) by real time PCR analysis. A substantial number of the top-ranking differentially expressed genes identified in OA bone are known to have roles in osteoblasts, osteocytes and osteoclasts. Many of these genes are targets of either the WNT or TGFβ/BMP signalling pathways. Other differentially expressed genes included WNT or TGFβ/BMP identified as differentially expressed in OA bone between females and males, consistent with our other data showing biochemical differences between males and females with OA.
Conclusions: The limitation of this work is that samples were taken at end stage disease. However, altered expression of sets of genes involved in bone turnover suggests a molecular basis for the altered bone remodelling observed throughout OA progression. These data may also in part explain the gender disparity observed in OA.

I-31 PATTERNS OF JOINT DISTRIBUTION IN HAND OSTEOARTHRITIS: GENETIC CONSIDERATIONS

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Purpose: 1. To examine the pattern of joint distribution in radiographic hand osteoarthritis (HOA) development in apparently healthy human population and to assess contribution of sex and age on it. 2. To test the effect of the various joint degeneration features to Kellgren-Lawrence (K-L) score of the different joints and in total. 3. To evaluate contribution of the putative genetic factors to inter-individual variation of HOA. 4. To test the association of the extracellular pyrophosphate channel genes (specifically ENPP1) with the variation of the radiographic HOA in the above population.
Methods: The study sample was comprised of Chuvashains (Europeans living along Volga river in Russia): 1,200 individuals of both sexes, with age range between 19 and 90 years. HAO development was assessed radiographically for 15 joints of each hand according to K-L grading system, with modifications. First, using extensive statistical analysis we examined the rate and pattern of age related changes of each type of joints (DIP, PIP etc) and each finger separately in both sexes. We also assessed quantitatively the relative contribution of the specific joint degeneration characteristics (such as osteophytes, OS; joint space narrowing JSN; subchondral cysts, SC; etc) to the K-L grade for each type of joint and in total. Next, 12 DNA polymorphisms, located within and in vicinity of ENPP1 were tested in 126 nuclear families with 574 adult individuals. Family-based association analysis was conducted between these markers and total K-L score of HOA.
Results: As expected, we found very strong association of HAO with age, with only minor differences between the sexes. However, the rate of the K-L scores appearance varied substantially between the fingers and especially between the joints. The fifth finger and DIP joints showed the highest *vs* the first finger and IP1 joint the lowest rate of degeneration respectively. Cluster analysis for rows of joints and joint groups of both hands revealed that symmetry is the most common pattern of interrelationships between rows of joints as well as between the fingers, when the later were compared as entire units. The contribution of the different joint degeneration characteristics to K-L score was very different: OS made the major contribution to all types of joints and in total, ranging from 50% to 80%. SC was the second predictor, contributing between 10% to 23% to K-L score. The rest made only minor contribution. Model-based statistical genetic analysis of the total K-L score, adjusted for age and sex, showed highly significant contribution of the familial factors explaining >25% of the trait variation. The model fitting analysis also strongly supported the hypothesis of the genetic effect on the OS score variation alone. Testing for the association with DNA polymorphisms in ENPP1 gene consistently showed significant association ($p < 0.05$–0.001) with this gene, suggesting its involvement in HOA development.
Conclusions: The present data show that the rate of HOA development associated with age varied significantly between the different types of joints and fingers, with osteophytosis as a major predictor of OA at all joints. Our study highlights significant genetic contribution to interindividual variability of HOA and clearly suggest ENPP1 gene as an important genetic factor in the pathogenesis of idiopathic osteoarthritis.

I-32 PHENOTYPING EROSIVE OSTEOARTHRITIS OF THE HAND

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Purpose: Erosive osteoarthritis of the hand (EOA) is characterised by an aggressive clinical course and the presence of erosive radiographic changes. Over time, EOA has been considered as a variant of OA, a subset of OA, a severe form of OA, an inflammatory phase of OA, and an entity distinct from OA. Our aim is to phenotype EOA in order to demonstrate that it is a distinctive subset of hand OA.
Methods: We examined studies analysing characteristic features of EOA, including clinical, radiographic and laboratory aspects. The diagnosis of EOA was usually based on ACR clinical criteria for hand OA and radiographic aspects of articular erosions, at least two in two different IPs and with the exclusion of MCPs. The definition of erosion is still a matter for debate. Most frequently, lesions begin at the central portion of the joint as a sharply marginated defect, usually preceded by joint narrowing. Progression commonly leads to the so-called 'gull-wing' deformity due to marginal sclerosis and osteophytes of the distal side of the joints, while the proximal side is centrally eroded or collapsed and thinned. Subchondral pseudocysts may be observed on the proximal side. Another

characteristic erosion, found especially in PIP, is the so-called "saw tooth" appearance.
Results: Although the diagnosis of EOA is based on radiographic erosions, some clinical features may lead to a suspicion of EOA. According to the definition of "inflammatory OA", abrupt onset of pain, swelling, redness, warmth and limited function of IP joints are common. Sometimes the same features may be observed in non-EOA cases, but usually at the disease onset, during the first year. Characteristic of EOA is the throbbing paresthesias of the fingertips. EOA may lead to joint deformities, some indistinguishable from those of non-EOA, such as lateral subluxations and Heberden's and Bouchard's nodes, while others are seen almost exclusively in EOA, such as instability and ankylosis of DIP and PIP and, rarely, opera-glass deformity.
Concerning laboratory investigations, ultrasensitive CRP has been proposed as marker of the disease activity. Some OA markers, such as the CTX I, were found to be increased in the serum and urine in EOA in comparison with nodal non-EOA. In another recent study, serum levels of myeloperoxydase (MPO) and, at lesser extent, Coll2−1NO2, were elevated in EOA in comparison with non-EOA.
Pattrick et al observed an increased frequency of the HLA-A1B8 and MZ a1-antitrypsin phenotypes in patients with nodal OA. Among these, patients with EOA had an increased frequency of the MZ a1-antitrypsin phenotype (30 versus 9%). We found that HLA DRB1*011 was associated with EOA, in comparison with non-EOA and reference populations. Stern et al reported an association between EOA and a genomic region containing the interleukin-1b (Il-1b) 5810 single nucleotide polymorphism, thus supporting a potential role for Il-1 in the pathogenesis of this severe phenotype of hand OA.
In comparison with nodal OA, clinical aspects of EOA may sometimes be indistinguishable, although EOA is characterized by more frequent inflammatory episodes involving several joints simultaneously and may persist for many years, while nodal generalized OA exhibits its flares mainly at onset of the involvement of each joint, in a 'stuttering' onset polyarthropathy of DIPs and PIPs which resembles a 'monoarthritis multiplex'. Furthermore, instability and ankylosis of IPs are seen almost exclusively in EOA.
Conclusions: According to the recent EULAR recommendations for the diagnosis of hand OA, EOA may be considered "as subset of hand OA, characterized by radiographic erosions targeting IP joints which may progress to marked bone and cartilage attrition, instability and bony ankylosis. Typically it has an abrupt onset; marked pain and functional impairment; inflammatory symptoms and signs (stiffness, soft tissue swelling, erythema, paraesthesiae); mildly elevated CRP; and a worse outcome than non-EOA".

I-33 EXPERIMENTAL MODEL SYSTEMS TO ASSESS JOINT PAIN

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Purpose: The majority of patients suffering from osteoarthritis (OA) desire effective and safe pain relief. Despite the growing number of OA patients worldwide and the pressing need for efficacious analgesics, surprisingly little is known about the source and mechanisms of OA pain (for review see McDougall, 2006. Arthritis Res. Ther. 8: 220–229). One of the puzzling aspects of OA pain is the apparent disconnect between disease severity and patient reported pain levels. Animal models of OA provide a unique opportunity to investigate joint pain in an objective and empirical manner.
Methods: We have examined nociception in two animal models of OA viz. the Dunkin Hartley guinea pig model of spontaneous OA and the sodium monoiodoacetate rat model of chemically-induced OA. In the guinea pig model, the effect of age and joint destruction on joint nociceptor activity was determined by comparing micro-CT and histomorphological markers of knee joint pathology with single unit electrophysiological recordings from articular primary afferents. Nerve recordings were also performed on monoiodoacetate-induced OA rats as well as pain behaviour responses using hindlimb weight bearing and von Frey hair algesiometry. Pharmacological interventions were carried out in the rat model to help identify the chemical mediators involved in OA pain production and to discover novel pain therapeutics.
Results: Both animal models demonstrated heightened nerve activity at rest (spontaneous firing) and in response to mechanical rotation of the knee in the normal working range (non-noxious mechanical stimuli) and during noxious hyper-rotation. Disease severity in Dunkin Hartley guinea pig knees became progressively worse with advancing age and correlated with increased body mass; however, OA severity did not correlate with any of the objective electrophysiological measures of nociceptor activity. Peripheral administration of the neuropeptide antagonist VIP6−28 to monoiodoacetate-treated rat knees, reduced joint afferent sensitization (Schuelert & McDougall, 2006. Osteoarthr. Cart. 14: 1155–1162) and inhibited pain behaviour (McDougall et al., 2006. Pain 123: 98–105). In other experiments, local administration of a cannabinoid agonist attenuated afferent firing rate indicative of an anti-nociceptive effect in OA joints (Schuelert & McDougall, 2008. Arthritis Rheum. 58: 145–153).
Conclusions: These studies show that OA leads to peripheral sensitization of knee joint sensory nerves and this heightened neural activity is the physiological basis of joint pain. The data also provide the first objective evidence that disease severity is a poor indicator of joint pain. Moreover, we have identified VIP6−28 and locally administered cannabinoids as potential treatments for OA pain. Future studies using these and other OA models are required to elucidate further the neurophysiological processes responsible for generating joint pain so that novel therapeutics may be developed allowing OA patients to lead a pain-free life.

I-34 BEHAVIORAL METHODS FOR ASSESSING OA AND PAIN IN MOUSE MODELS

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Purpose: Osteoarthritis (OA) is a major musculoskeletal disorder that manifests with functional limitation and pain. In the United States, the incidences of OA are projected to double by 2020 primarily due to increased obesity. Although clinicians have concentrated upon treating the symptoms of OA and, in some cases, by advising changes in diet, various behavioral abnormalities may accompany the disease. This presentation will describe some methods for examining abnormal behaviors in mouse models of OA.
Methods: Neuromuscular status can be evaluated through behavioral tests of reflexes, posture, grip-strength, pole-climbing/walking, gait, and sensorimotor skills. Pain sensitivities are examined by tests of mechanical and thermal sensitivities. Anxiety-like behaviors are analyzed by responses in the zero maze, light-dark box, open field, and novelty-suppressed feeding tests, whereas depressive-like behaviors are studied by forced swim, tail suspension, and anhedonia.
Results: In a genetic model of OA, *Col9a1* mice showed knee joint degeneration and were deficient in sensorimotor responses and displayed heightened mechanical sensitivities. In a diet-induced model of OA, C57BL/6 mice fed a high fat diet also had knee joint changes. These animals presented with some deficiencies in sensorimotor skills and they displayed anxiety-like behaviors in the zero maze, as well as analgesia in the hot plate but appeared normal in the tail flick test.
Conclusions: As anticipated, these mouse models of OA show alterations in sensorimotor responses and pain sensitivities. However, the animals also display additional behavioral abnormalities. Clinicians should be aware that OA patients may present with similar comorbidities and treatment of these behaviors should be considered part of the regimen for OA therapy.

I-35 THE IDENTIFICATION OF SUSCEPTIBILITY GENES FOR THE ONSET AND PROGRESSION OF OSTEOARTHRITIS

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Purpose: To identify new osteoarthritis susceptibility genes for the onset and progression of Osteoarthritis.
Methods: A genome-wide linkage scan and combined linkage association analysis was applied to 179 affected siblings and 4 trios with generalized osteoarthritis (The GARP study). We tested for confirmation by association in 3 additional independent OA populations.
Results: Suggested evidence for linkage in the GARP study was observed on chromosome 14q32.11 (LOD = 3.03, $P = 1.9 \times 10^{-4}$). Genotyping tagging SNPs covering three important candidate genes revealed a common coding variant (rs225014; Thr92Ala) in the iodothyronine-deiodinase enzyme type 2 (D2) gene (*DIO2* [MIM 601413]*)* which significantly explained the linkage signal ($P = 0.006$). Confirmation and replication by association in the additional osteoarthritis studies indicated a common *DIO2* haplotype, exclusively containing the minor allele of rs225014 and common allele of rs12885300, with a combined recessive odds ratio of 1.79, 95% confidence interval [CI] 1.37−2.34 with $P = 2.02 \times 10^{-5}$ in females cases with advanced/symptomatic hip osteoarthritis. The gene product of this DIO2 converts intracellular prohormone-3,3′,5,5′-tetraiodothyronine (T4) into the active thyroid hormone